

WHAT IS CLAIMED IS:

1. A method for testing a compound for activity as an agonist or antagonist of a calcium channel, comprising the steps of:

5 (a) contacting a cell expressing a functional voltage-gated calcium ion channel and a functional potassium ion channel with a solution having a potassium concentration where the membrane potential of the cell is modulated without fully depolarizing the cell;

(b) simultaneous to or subsequent to step (a), contacting the cell with (i) a substance of interest and (ii) an ion or molecule capable of entering the cell through a functional calcium channel;

10 (c) depolarizing the cell membrane of the cell;

(d) detecting the channel mediated ion flux into the cell; and

(e) comparing the ion flux thus detected from step (d) to an ion flux produced in a control experiment, wherein the control experiment comprises subjecting a separate cell to the steps (a), (b)(ii), (c) and (d), but not step (b)(i);

15 where a difference in ion flux detected in step (d) and the control experiment indicates that the substance of interest is an agonist or antagonist of a calcium channel.

2. A method of identifying state-dependent antagonists of a voltage-gated calcium ion channel comprising:

20 (a) providing a divided tissue culture plate comprising individual compartments, where at least two of the individual compartments contain living eukaryotic cells that express a plurality of functional voltage-gated calcium ion channels and functional potassium channels on their plasma membranes, the cytoplasm of the cells comprising an ion-sensitive fluorescent indicator compound;

25 (b) adjusting the membrane potential of the cells by altering extracellular potassium concentration in at least one of the compartments containing the cells;

(c) adding a substance of interest to at least one of the individual compartments containing the cells;

30 (d) depolarizing the cells in the at least two compartments containing cells, wherein at least one compartment is subjected to step (c), test group, and at least one compartment is not subjected to step (c), control group;

(e) detecting the ion flux into the cells of step (d); and

(f) comparing the ion flux into the cells of the test group with the cells of the control group;

where if the value of ion flux in the test group cells is lower than the control group cells, the substance is an antagonist of the voltage-gated calcium ion channel.

3. The method of claim 2, wherein the divided tissue culture plate is a
5 multiwell tissue culture plate comprising at least two wells.

4. The method of claim 3, wherein the multiwell tissue culture plate
comprises 12, 24, 96, 384, 1,536, or 3,456 wells.

10 5. The method of claim 2, wherein at least 10 substances are tested in a 24
hour period.

6. The method of claim 2 where the cells are selected from the group
consisting of: L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), HEK293
15 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650),
COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC
CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5
(ATCC CCL 171), CPAE (ATCC CCL 209), Saos-2 (ATCC HTB-85), ARPE-19 human retinal
pigment epithelium (ATCC CRL-2302), GH3 cells, TReX-292 cells, T-ReX-CHO cells, and
20 primary cardiac myocytes.

7. The method of claim 2, where the cells are HEK293 cells stably
transfected to express the alpha-1C subunit of the voltage-gated calcium ion channel and Kir 2.3
inward-rectifying potassium channel.

25 8. The method of claim 2 wherein the fluorescent indicator compound is
selected from the group consisting of fluo-3, fura-2, fluo-4, fluo-5, calcium green-1, Oregon
green, 488 BAPTA, SNARF-1, and indo-1.

30 9. The method of claim 2, wherein the substance is identified as an
antagonist when the current flow into the cells of the test group is lower than the current flow
into the cells of the control group.

35 10. The method of claim 2, wherein the detecting step (e) employs a
fluorescence or luminescence indicator device.

11. The method of claim 2, wherein the detecting step (e) employs a FLIPR or VIPR device.

5 12. A method of identifying state-dependent antagonists of a voltage-gated calcium ion channel comprising:

(a) providing a divided tissue culture plate comprising individual compartments, where at least two of the individual compartments contain living eukaryotic cells that express a plurality of functional alpha 1C calcium ion channels and functional Kir 2.3 inward rectifying potassium channels on their plasma membranes, the cytoplasm of the cells comprising an ion-sensitive fluorescent indicator compound;

10 (b) adjusting the membrane potential of the cells by altering extracellular potassium concentration in at least one of the compartments containing the cells;

(c) adding a substance of interest to at least one of the individual compartments containing the cells;

15 (d) depolarizing the cells in the at least two compartments containing cells, wherein at least one compartment is subjected to step (c), test group, and at least one compartment is not subjected to step (c), control group;

(e) detecting the ion flux into the cells of step (d); and

20 (f) comparing the ion flux into the cells of the test group with the cells of the control group;

where if the value of ion flux in the test group cells is lower than the control group cells, the substance is an antagonist of the voltage-gated calcium ion channel.

25 13. The method of claim 12 further comprising comparing ion flux in the test group with that in a second test group, the second test group comprising cells subjected to steps (b) and (c), but whose membrane potentials have been adjusted to a value different than that of the test group cells;

30 where if the value of ion flux in the test group cells is different than the value of ion flux in the second test group cells, then the substance possesses a state-dependent potency on the voltage-gated calcium ion channel.

14. A method of identifying antagonists possessing state-dependent potency for a voltage-gated calcium ion channel comprising:

- 5 (a) providing a divided tissue culture plate comprising individual compartments, where at least two of said individual compartments contain living eukaryotic cells that express a plurality of functional voltage-gated calcium ion channels and functional potassium channels on their plasma membranes, the cytoplasm of said cells comprising an ion-sensitive fluorescent indicator compound;
- (b) adjusting the membrane potential of a first group of cells and a second group of cells by altering extracellular potassium concentration in the individual compartments containing said first and second group of cell, wherein the membrane potential of the second test group cells is lower than the value of the first test group cells;
- 10 (c) adding a substance of interest to the individual compartments containing the first and second groups of cells;
- (d) depolarizing the cells in the at least two compartments containing cells;
- (e) detecting the ion flux into the cells of step (d); and
- (f) comparing the ion flux in the cells of the first test group with the cells of
- 15 the second test group;
- where if the value of ion flux in the first test group cells is different than the value of ion flux in the second test group cells, then the substance possesses state-dependent potency for said voltage-gated calcium ion channel.